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CHARACTERIZATION OF *SPIRULINA* BIOMASS  
FOR CELSS DIET POTENTIAL

MAHASIN G. TADROS  
ALABAMA A&M UNIVERSITY  
NORMAL AL 35762

AUGUST 1993

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FOR *CELSS* DIET POTENTIAL**

**PRINCIPAL INVESTIGATOR  
ALABAMA A&M UNIVERSITY  
NORMAL , AL 35762**

**MAHASIN G. TADROS, PH.D**

**TECHNICAL MONITOR  
NASA / AMES  
MOFFET FIELD, CA 94035**

**ROBERT D. MACELROY, PH.D**

**NASA COOPERATIVE AGREEMENT**

***CELSS* PROGRAM**

**AUGUST 1993**



## **TABLE OF CONTENTS**

	<b>Page</b>
<b>ACKNOWLEDGEMENT AND PERSONNEL</b>	<b>i</b>
<b>SUMMARY</b>	<b>ii</b>
<b>INTRODUCTION AND BACKGROUND</b>	<b>1</b>
<b>Objectives</b>	<b>3</b>
<b>Significance</b>	<b>3</b>
<b>MATERIALS AND METHODS</b>	<b>3</b>
<b>Culturing</b>	<b>3</b>
<b>Apparatus</b>	<b>4</b>
<b>Analysis and Calculations</b>	<b>5</b>
<b>EXPERIMENTAL DESIGN</b>	<b>9</b>
<b>RESULTS AND DISCUSSION</b>	<b>10</b>
<b>CONCLUSION</b>	<b>32</b>
<b>REFERENCES</b>	<b>33</b>



## LIST OF FIGURES

	Page
<b>FIGURE 1: Growth Characteristics Of <i>Spirulina maxima</i> Maintained In Complete Growth Medium, In Steady State Cultures (Light Intensity, 30 <math>\mu\text{E m}^{-2} \text{ s}^{-1}</math>).</b>	11
<b>FIGURE 2: Growth Characteristics Of <i>Spirulina maxima</i> Maintained In Complete Growth Medium, In Steady State Cultures (Light Intensity, 100 <math>\mu\text{E m}^{-2} \text{ s}^{-1}</math>).</b>	12
<b>FIGURE 3: Chemical Composition Of <i>Spirulina maxima</i> Maintained In Complete Growth Medium, In Steady State Cultures (Light Intensity, 30 <math>\mu\text{E m}^{-2} \text{ s}^{-1}</math>, 3a; 100 <math>\mu\text{E m}^{-2} \text{ s}^{-1}</math>, 3b).</b>	13
<b>FIGURE 4: Growth Characteristics Of <i>Spirulina maxima</i> Maintained In Growth Medium, Of Different Concentrations Of Nitrate-Nitrogen, In Steady State Cultures (Light Intensity, 30 <math>\mu\text{E m}^{-2} \text{ s}^{-1}</math>).</b>	17
<b>FIGURE 5: Growth Characteristics Of <i>Spirulina maxima</i> Maintained In Growth Medium Of Different Concentrations Of Nitrate-Nitrogen, In Steady State Cultures (Light Intensity, 100 <math>\mu\text{E m}^{-2} \text{ s}^{-1}</math>).</b>	18
<b>FIGURE 6: Chemical Composition Of <i>Spirulina maxima</i> Maintained In Growth Medium Of Different Concentrations Of Nitrate-Nitrogen, In Steady State Cultures (Light Intensity, 30 <math>\mu\text{E m}^{-2} \text{ s}^{-1}</math>, 6a; 100 <math>\mu\text{E m}^{-2} \text{ s}^{-1}</math>, 6b).</b>	19

<b>FIGURE 7:</b>	<b>Growth Characteristics Of <i>Spirulina maxima</i> Maintained Of Different Concentrations Of Phosphate, In Steady State Cultures (Light Intensity, 30 uE m<sup>-2</sup> s<sup>-1</sup>).</b>	<b>23</b>
<b>FIGURE 8:</b>	<b>Chemical Composition Of <i>Spirulina maxima</i> Maintained In Growth Medium Of Different Concentrations Of Phosphate, In Steady State Cultures (Light Intensity, 30 uE m<sup>-2</sup> s<sup>-1</sup>).</b>	<b>24</b>
<b>Figure 9:</b>	<b>Growth Characteristics Of <i>Spirulina maxima</i> Maintained In Growth Medium Of Different Concentrations Of Sodium Chloride, In Steady State Cultures (Light Intensity, 30 uE m<sup>-2</sup> s<sup>-1</sup>).</b>	<b>27</b>
<b>FIGURE 10:</b>	<b>Growth Characteristics Of <i>Spirulina maxima</i> Maintained In Growth Medium Of Different Concentrations Of Sodium Chloride, In Steady State Cultures (Light Intensity, 100 uE m<sup>-2</sup> s<sup>-1</sup>).</b>	<b>28</b>
<b>FIGURE 11:</b>	<b>Chemical Composition Of <i>Spirulina maxima</i> Maintained In Different Concentrations Of Sodium Chloride, In Steady State Cultures (Light Intensity, 30 uE m<sup>-2</sup> s<sup>-1</sup>, 11a; 100 uE m<sup>-2</sup> s<sup>-1</sup> 11 b).</b>	<b>29</b>



## LIST OF TABLES

	Page
TABLE 1: Growth Characteristics Of <i>Spirulina maxima</i> Maintained In Complete Growth Medium, In Steady State Cultures (Light Intensity, 30 uE m <sup>-2</sup> s <sup>-1</sup> )	14
TABLE 2: Growth Characteristics Of <i>Spirulina maxima</i> Maintained In Complete Growth Medium, In Steady State Cultures (Light Intensity, 100 uE m <sup>-2</sup> s <sup>-1</sup> )	15
TABLE 3: Growth Characteristics Of <i>Spirulina maxima</i> Maintained In Growth Medium Of Different Concentrations Of Nitrate-Nitrogen, In Steady State Cultures (Light Intensity, 30 uE m <sup>-2</sup> s <sup>-1</sup> )	20
TABLE 4: Growth Characteristics Of <i>Spirulina maxima</i> Maintained In Medium Of Different Concentrations Of Nitrate-Nitrogen, In Steady State Cultures (Light Intensity, 100 uE m <sup>-2</sup> s <sup>-1</sup> )	21
TABLE 5: Growth Characteristics Of <i>Spirulina maxima</i> Maintained In Medium Of Different Concentrations Of Phosphate, In Steady State Cultures (Light Intensity, 30 uE m <sup>-2</sup> s <sup>-1</sup> )	25
TABLE 6: Growth Characteristics Of <i>Spirulina maxima</i> Maintained In Medium Of Different Concentrations Of Sodium Chloride, In Steady State Cultures (Light Intensity, 30 uE m <sup>-2</sup> s <sup>-1</sup> )	30
TABLE 7: Growth Characteristics Of <i>Spirulina maxima</i> Maintained In Medium Of Different Concentrations Of Sodium Chloride, In Steady State Cultures (Light Intensity, 100 uE m <sup>-2</sup> s <sup>-1</sup> )	31

## **ACKNOWLEDGEMENT**

Appreciation is extended to *NASA-AMES* for their support of this project.

As the *NASA Technical Monitor*, *Dr. Robert MacElroy* has given invaluable guidance and assistance to the project.

To him, we are especially grateful.

## **PERSONNEL**

The following personnel have been involved in this project :

Mahasin G . Tadros, Ph.D., P.I.

Woodrow Smith , M.S.

Beverly Joseph, B.S.

Janelle Phillips, B.S.

## SUMMARY:

Cyanobacteria, Spirulina maxima as a biogenerative photosynthetic and an edible alga for the space craft crew in a CELSS, was evaluated in an effort to increase the growth rate, biomass, yield, and chemical analysis in continuous cultures. The cell characteristics were determined for cultures maintained at steady state with respect to the substrate concentration.

The productivity increased in experiments exposed to low light ( $30 \text{ uE m}^{-2} \text{ s}^{-1}$ ). Oxygen evolved and protein production were higher in cultures exposed to low light intensity. There was a relationship between nitrate concentration and the yield of the culture. Increasing the concentration of nitrate in the growth medium up to 20 mM was enough to produce a culture having the same chemical composition as that of complete medium. High light was inhibiting the yield of the culture. Increasing the concentration of phosphate beyond 1mM did not improve the yield of the culture. Increasing the concentration of sodium chloride in the growth medium did not affect the growth of the alga up to 0.1M but beyond that the culture started to be stressed. The response to stress appeared in high production of total carbohydrate on the expense of protein production. The oxygen production was also higher in cultures stressed with sodium chloride.



## INTRODUCTION AND BACKGROUND:

Pursuit of our national goals in space will eventually require man's longduration tenancy of celestial vehicles and planetary bases. Requirments for life support could be met through expenditure of stored supplies and by regeneration and reuse of the waste products of human metabolism. The logistics necessary for regeneration for extended space missions are well documented.

The use of biological components Controlled Ecological Life Support System (CELSS) program as subsystems for the revitalization of air, waste processing, and for the production of food has been proposed for the long term-space flight(MacElroy, Bredt, 1985). Studies of biogenerative life support systems for use in space indicated that they are scientifically feasible. Support of a crew in space, whether in an orbiter or on the surface of a planetary body requires that oxygen, potable water and food be supplied and that waste material be removed. Employment of photosynthetic organisms (higher plansts, green algae, cyanobacteria) allows biomass production from relatively simple componants which are readily recycled in a CELSS system, namely carbon dioxide, minerals ( $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ ,  $\text{K}^+$ ,  $\text{Na}^+$ , etc.) and micronutrients.

The primary source of all man's food and organic raw mateials is solar energy. Conventional food soruces consist of higher plants and animals. Unconventional food sources for human consumption are photosynthetic algae and bacteria and non-photosynthetic bacteris, yeasts and fungi. Conventional food sources are highly palatable, but require a long time to produce. The photosynthetic energy efficiency of higher plants is less than 3%. Algae, on the other hand, grow rapidly; their metabolism can be controlled; they produce a high ratio of edible to nonedible biomass; and their gas-exchange characterisitcs are compatable with human requiremnts. In many respects, algae would be ideal plant components for a biologically-based Closed Life Support System, since they are eminently suited to the closely coupled funtions of food production and atmosphere regeneration.

The semi-microscopic blue-green algae (Cynobacteria: Spirulina) occupy a unique taxonomic position, since they combine an autorophic mode of growth which is common to eukaryotic plant

cells with a metabolic system generally regarded as bacterial, rather than plant-like.

Photosynthetic efficiency is fundamentally important in both technological exploitation of photosynthesis and in deciding the molecular mechanism whereby light energy is converted to chemical energy. The continuous culture of photosynthetic cells provides the key to control over the growth conditions including the growth rate and growth limiting factor elucidation of how these conditions influence the photosynthetic systems in the cells (Pirt, 1983). Photosynthetic efficiency can be expressed as the ratio of free energy of the biomass produced: the amount of light energy absorbed or the number of Photons ( $h\nu$ ) required per mol  $O_2$  released or  $CO_2$  assimilated. The efficiency of photosynthesis is simply the rate by which chemical energy is stored in organic biomass divided by the supply rate of available light energy (Pirt, 1980). Pirt recommends that efficiencies be measured in growing cultures rather than by short term  $O_2$  evolution.

The photosynthetic efficiency (PE) is an expression of the growth yield from the energy source, light. Growth yield (Y) is defined as the biomass formed by light absorbed, and if K is the caloric value of the biomass, we have  $PE = KY$ . Increasing the efficiency of the yield of algal culturing in bioregenerative Life Support System, is one of the primary concerns of a CELSS.

In order to design and operate such a culture system, it is necessary to understand how the macroparameters of a culture system, e.g., productivity and efficiency are related to the physiological aspects of the algal culture.

Growth characteristics, as well as changes in partitioning, are needed to establish a data base for the integration of algal cultures into a CELSS program. This background could be obtained from studying the cyanobacteria: Spirulina maxima in batch and continuous cultures.

The results of continuous culture have been described partially in 1990 and this year 1993, reports (Tadros, 1990,1993).

## **Objectives:**

To evaluate the efficiency of the cyanobacteria: Spirulina maxima for the biomass, in response to changes in growth conditions such as, substrate concentrations, in steady state

## **Significance:**

Defining optimum growth conditions for high efficiency of the alga when used for regenerating sources needed for CELSS.

Development of CELSS relies; in part, on the ability to manipulate and control the organisms which are part of the system.

Biological regeneration of supplies consumed in CELSS.

Direct utilization of algae in space craft crew diet.

This project began in October 1990. The accomplishments during the period from October 1990 to February, 1993 are described in this report.

## **Material And Methods**

Culturing:

Organism: Cyanobacteria Spirulina maxima  
(UTEX LB 2342) was obtained from utex Algal Collection.

Growth Medium: Zarrouk medium (1966) was used for growth media.

Contamination: The standard plate-count method was used to determine the number of bacteria present in the culture. Aliquots were plated using a bent glass rod on an agar medium, which is prepared from Zarrouk (1966) medium enriched with the following ingredients:

Tryptone glucose yeast agar: Tryptone, 5.0; Yeast extract, 2.5g; Glucose, 1.0g; Agar, 15.0g; in 1L distilled water, pH 7.

0.1 ml filtrate was spread on the agar surface and incubated. Colonies were counted, dilution was made when necessary. Plates were

incubated at 30° C and counted after 48 hours or longer to detect all organisms.

Purification of spirulina culture: The original cultures of Spirulina were contaminated with bacteria. Different procedures were used to purify the cultures. However, the following procedure was the most successful one:

Cells were collected, filtered with 8mm filter (Gelman), washed with basal medium and homogenized. Cells were spread in a plate, exposed to UV 5 min ( 20 W UV lamp, 30 cm distance) and inoculated in test tube cultures containing the basal tubes, 10 tubes were bacteria free.

### **Apparatus:**

BRL's Airlift Fermenter was used for this project. (Tadros, 1990)

Two peristaltic pumps were used; one for feeding the fermenter with fresh medium, the second for removal of the overflow. The system was provided with ph controlling system. The ph of the medium remained almost unchanged at 9.3 to 9.4 in all systems by using 4N Sodium hydroxide or 1N HCL. The temperature of the cultures was maintained at 35° c.

### **Preculture:**

A batch cultures was kept parallel to fermenter, used for inoculating the reactor. The alga was precultured in small bottles (250 ml capacity) containing 100 medium at 35° C for 4 to 5 days. The cultures were illuminated with flourescent tubes at the intensity similar to that of the experiment. The aeration mixture as well was similar to that of the fermenter. The temperture was 35° C.

### **Principles of continuous culturing:**

A continous culture is a constant-volume cell culture system in which the rate of cell growth is controlled by the dilution rate of a nutrient-limited solution. The constant volume was maintained by ensuring that the rate of culture outflow equalled the inflow rate of the fresh medium. When steady- state conditions were reached, there existed a constant cell number and biomass within the vessel since the specific growth rate equalled the dilution rate. The medium was



kept refrigerated to avoid contamination. Steril medium was pumped from a reservoir into the vessel by a peristaltic pump. The outflow from the culture was collected in sterile bottles plugged with sterile cotton wool filters. The culture was assumed to be in steady state when the cell concentration remained constant for at least 96 hr. after the initial flow rate was adjusted.

In steady state culture:

Dilution rate ( $D$ )= $F/V$  where  $F$ = inflow rate (milliliters per hour) and  $V$  is culture volume (milliliters).

$D$ = growth rate

### **Analysis and Calculations:**

The following parameters were measured for the culture after it had reached its steady- state:

Harvesting of Cells: Cells were collected by filtration using filter paper 10mm pore size (Gelman). Cells were washed with buffer solution (pH 8), diluted to known volume and processed for further analysis.

Triplicate samples of the algal suspension were taken for each determination. The mean value of these triplicates was recorded. The following determination were carried out:

Total Chlorophyll: An aliquot from the culture was centrifuged for 2min. at 2000g. The precipitate was suspended in 5ml methanol for 5min in a water bath at 70° C, and thereafter centrifuged. The optical density of the supernatant was determined at 655nm.

Dry Weight Measurements (DW): A volume from the culture was filtered through a filter 10mm pore size, dried in previously dried, pre-weighed filter paper for 4 h at 80° C, and then weighed after cooling in a desiccator.

Ash-Free Dry Weight (AFDW): After recording the dryweight, the dried cells were ashed at 500° C for 2 hrs. Then the ash wt. was recorded. The difference between dry weight and ash weight gave the organic weight of the sample.

Total Carbohydrates: The anthrone sulphuric acid method was followed (Strickland, Pearson, 1972). The principle of this method is

the formation of a blue-green color which is the product by acid decomposition of the sugar. The anthrone reagent consist of 0.2g anthrone, 8ml ethyl alcohol, 10ml distilled water. Ten ml of the anthrone reagent was added to one ml of algal suspension (containing known weight of alga), heated in a water bath for seven minutes and cooled.

The blue-green color was measured by a spectrophotometer at wavelength of 620nm. The value of the reading was calted as micrograms of glucose from a standard curve for glucose which had been prepared by the same method.

Total lipids: Cellular lipids were solubilized by repeated extraction with methanol and methanol-chloroform (1: 1), then phase separated after adjustment of the solvent ratios to 10:9:9 (methanol:chloroform:water, v/v) (Bligh and Dyer 1959). The chloroform phase was collected, evaporated to dryness under N<sub>2</sub>, and the weight of the lipid extract divided by the ash free dry weight of the original sample.

Total Nitrogen and Protein (Kjeldahl): One ml of algal suspension containing a given weight was digested in a Kjeldahl digestion flask containing 0.3g selenium mixture and one ml sulfuric acid. When the contents became colorless, they were transferred to the Kjeldahl apparatus with 10ml of 50% sodium hydroxide solution. A strong current of steam in a 100ml flask containing 5ml of 2% boric acid solution and 4 drops of indicator. The indicator was composed of 0.016g methyl red and 0.83g bromocresol green in 100ml of alcohol. The distillate in the boric acid solution was back titrated with 0.1 M sulphuric acid, until the color of the indicator turned plae pink. A blank sample was done for each series of nitrogen estimation, using D.W. The value of the reading was calculated in ug N, from a standard curve for nitrogen source as ammonium sulfate, which had been treated by the same method. Total potein was calculated from total N X 6.25.

Total Phosphorus: The supernatant residue (after evaporation) and algal residue, was heated with perchloric acid to liberate phosphours as inorganic phosphorus (Strickland and Parsons, 1972). The principle of this method was that the phosphorus reacts with ammonium paramolybdate to form the blue molybdophosphate complex, the absorbance of which is measured with a spectrophotometer at 840-880 nm against an appropriate blank. The

reading of sample was calculated from the standard curve prepared by using  $\text{KH}_2\text{PO}_4$ .

#### Elemental Analysis:

Algal sample were analyzed for carbon, hydrogen and nitrogen by Galbraith Laboratories, Inc., Tennessee. The oxygen content of the ash-free biomass was determined by the difference after subtraction of the C, H, and N contents. Perkin-Elmer 2400 Elemental Analyzer was used for the analysis. Values of analysis were reported as percentage of weight.

All of the above analytical data were related to the organic weight of the algae. All tests were performed in triplicate.

#### Light Measurements:

Light incident and absorbed by the culture was measured with a Li Cor Li - 185 Quantum sensor probe (unit =  $\mu\text{E m}^{-2} \text{ s}^{-1}$ ).

Measurements of optical density within the main culture were also taken by measuring light (I) transmitted through the culture and light incident ( $I_0$ ) on the culture.  $\log I/I_0$  (optical density) correlated very well with dry weight.

#### Absorbed light by the culture:

Volume of culture (Fermente) = 2000 ml

Illuminated area = 528  $\text{cm}^2$

( Area of fermenter exposed to light irradiation)

$a/v$  = ratio of illuminated area (a) to culture volume (v).

$I_a$  (absorbed light) = Intensity of light incident on culture - Intensity of light out of culture

$I_a \times a/v$  = absorbed light by culture

#### Measurement of Oxygen Evolution:

Samples were taken from the fermenter and evaluated for oxygen production using a Clark-Oxygen electrode. Oxygen evolution was

measured under the same conditions of the fermenter. Oxygen evolved was recorded.

Productivity: (g/h)

Productivity is defined as the product of dry weight (g/l) of the culture and the overflow rate (l/h).

Light efficiency:

Light efficiency was calculated using the absorbed light intensity ( $\text{cal/cm}^2/\text{l}$ ) and biomass productivity, converted from g/h to cal/h using heat of combustion.

Growth yield:

Yields are grams of dry algae per unit illuminated surface area and per unit total culture volume.

Efficiency:

Over-all conversion of electrical energy into algal cells: percent efficiency is based on heat of combustion.

Heat of combustion:

The heat of combustion of the culture was calculated from the elemental composition using the empirical formula according to Fontes et al. (1989).

## **EXPERIMENTAL DESIGN:**

### **I. Growth Characteristics Of Spirulina Maintained In Complete Growth Medium, At Steady State.**

Experiments were incubated in two light intensities 30 and 100  $\mu\text{E m}^{-2} \text{ s}^{-1}$ .

### **II. Growth Characteristics Of Spirulina Maintained In Growth Medium Of Different Concentrations Of Nitrate-Nitrogen, At Steady State.**

Experiments were incubated in two light intensities 30 and 100  $\mu\text{E m}^{-2} \text{ s}^{-1}$ .

### **III. Growth Characteristics Of Spirulina Maintained In Growth Medium Of Different Concentrations Of Phosphate-Phosphorus, At Steady State.**

Experiments were incubated in two light intensities 30 and 100  $\mu\text{E m}^{-2} \text{ s}^{-1}$ .

### **IV. Growth Characteristics of Spirulina Maintained in Growth Medium of Different Concentrations Of sodium chloride, at steady state.**

Experiments were incubated in two light intensities 30 and 100  $\mu\text{E m}^{-2} \text{ s}^{-1}$ .

## **RESULTS And DISCUSSION:**

### **I. Growth Characteristics Of Spirulina Maintained In Complete Growth Medium, At Steady State.**

Results are presented in Table 1 and 2 and Figures 1, 2, and 3.

. The dilution rate was almost stable, (Figure 1a and 2a) in the control growth medium (30 mM Nitrate-N) in both light intensities, however the dilution rate was higher in culture exposed to high light ( $100 \text{ uE m}^{-2} \text{ S}^{-1}$ ).

. The total dry weight was higher in cultures exposed to low light intensity (Figure 1a) than to cultures exposed to high light intensity (Figure 2b).

. The total uptake of nitrogen and phosphorus is the same in both experiments.

. The chemical composition of both cultures is represented in (Figure 3a and b) The total protein was lowered by increasing the light intensity (Figure 3b).

. The oxygen evolved by the culture was higher in low light than in high light (Table 1 and 2).

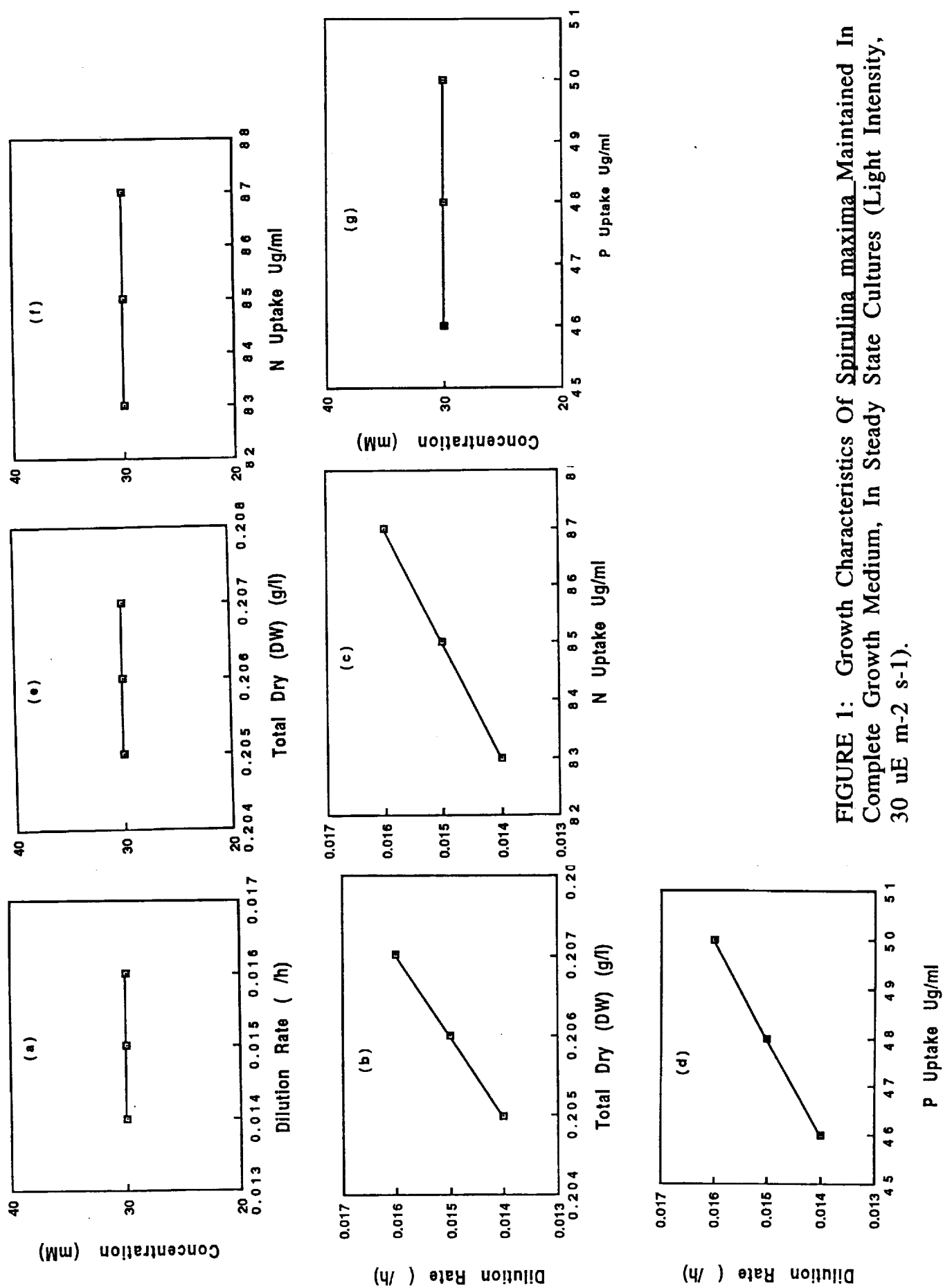


FIGURE 1: Growth Characteristics Of *Spirulina maxima* Maintained In Complete Growth Medium, In Steady State Cultures (Light Intensity, 30 uE m<sup>-2</sup> s<sup>-1</sup>).

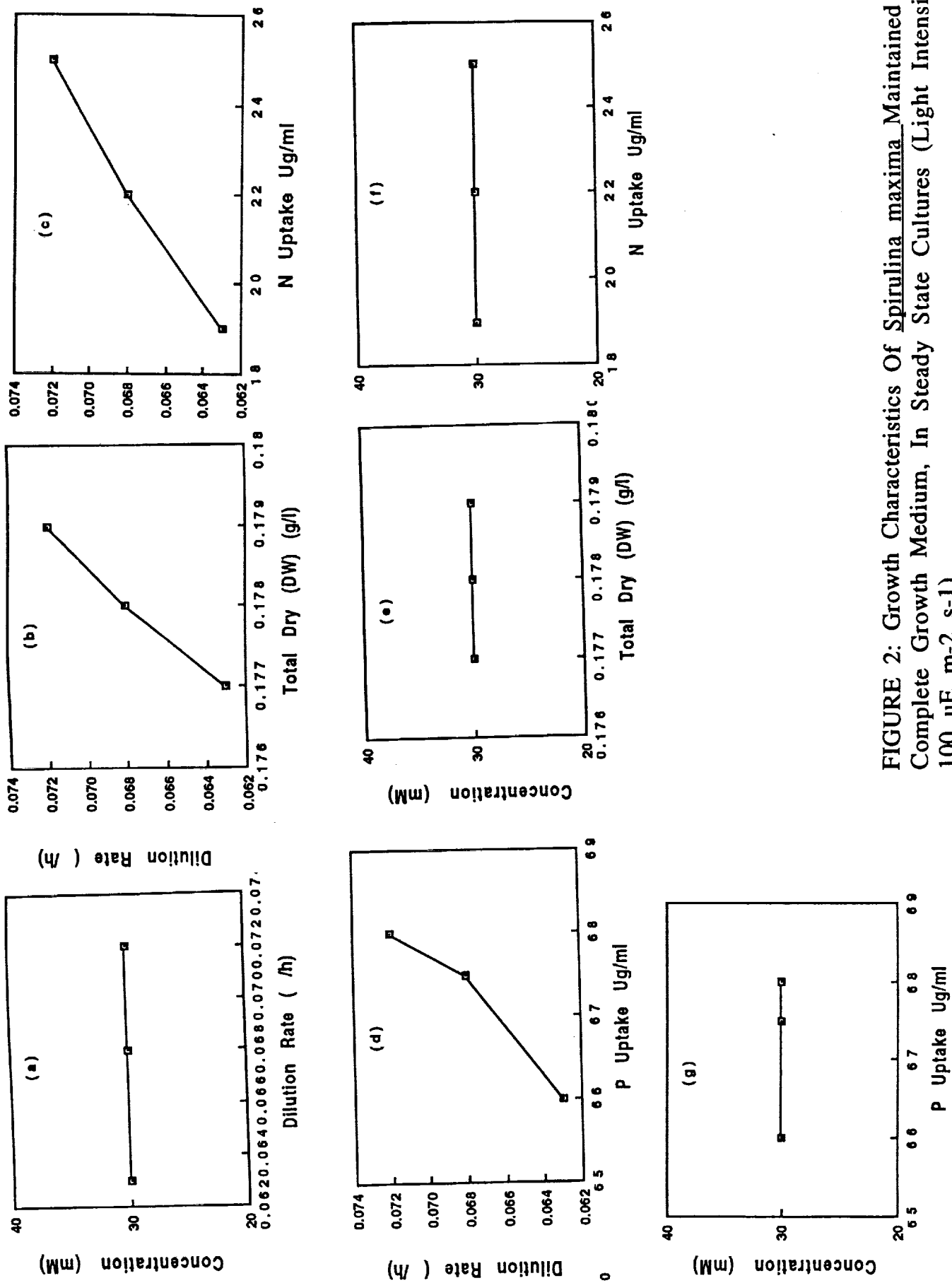


FIGURE 2: Growth Characteristics Of *Spirulina maxima* Maintained In Complete Growth Medium, In Steady State Cultures (Light Intensity, 100  $\mu\text{E m}^{-2} \text{s}^{-1}$ ).



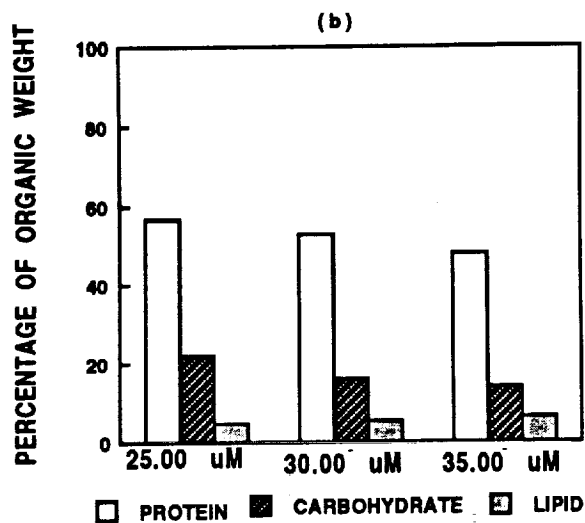
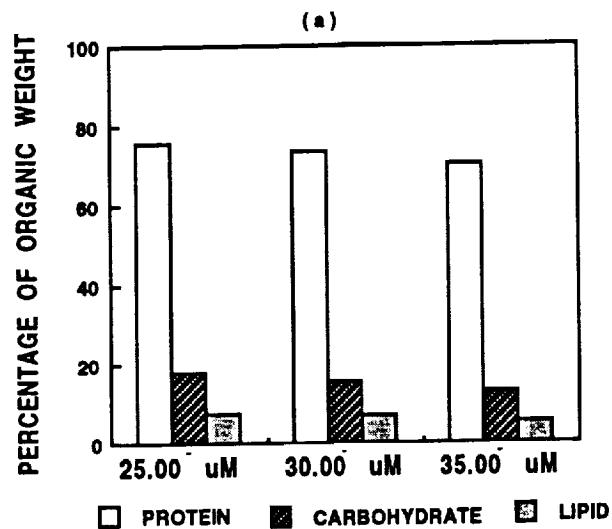


FIGURE 3: Chemical Composition Of Spirulina maxima Maintained In Complete Growth Medium, In Steady State Cultures (Light Intensity, 30 uE m<sup>-2</sup> s<sup>-1</sup>, 3a; 100 uE m<sup>-2</sup> s<sup>-1</sup>, 3b).



TABLE 2: Growth Characteristics Of Spirulina maxima Maintained In Complete Growth Medium, In Steady State Cultures (Light Intensities, 100 uE m-2 s-1).

O.D.	ml	Dilu tion Rate ( /h)	Tot. Dry (DW) (g/l)	Produc tivity (g/h/l)	Chloro phyll (mg/l)	Inc ident Light (cal/ cm/h)	Abso rbed Light a/v (cal/ cm/h)	Growth Yield (g dw /cal)	Photo synth etic % Dw/h	Oxygen Evolved uM O2	% Organic wt. (afdw)			N Uptake Ug/ml	P Uptake Ug/ml
											Protein	Carbohydrate	Lipid		
0.3	30	0.072	0.179	0.0128	4.571	0.4935	0.4274	0.0299	17.64	600	60.20	5.37	4.90	25	68

## **II. Growth Characteristics Of Spirulina Maintained In Growth Medium Of Different Concentrations Of Nitrate-Nitrogen, At Steady State.**

Results are presented in Tables 3 and 4, and Figures 4, 5, and 6.

- . Increasing the concentration of nitrate-nitrogen did not effect the dilution rate or dry weight (Figure 4a and 4b). The nitrogen and phosphorus uptake were also lowered with increasing the nitrate concentration.
- . At high light intensity ( $100 \text{ uE m}^{-2} \text{ s}^{-1}$ ) the dilution rate was higher than low light intensity ( $30 \text{ uE m}^{-2} \text{ s}^{-1}$ ) (compare figure 4a and 5a).
- . Increasing the concentration of nitrate-N beyond 10mM, leads to bleaching the culture.
- . The uptake of nitrate per ml was high in case of low light intensity than that of high light intensity (Fig 4b and 5b).
- . The total protein was increased with increasing the concentration of nitrate-N in low and high light intensity (Figure 6a and 6b)  
Increasing the light intensity inhibited the growth of the culture beyond 10mM nitrate (Figure 6b).
- . The total oxygen increased with increasing the nitrate concentration (Table 3 and 4).

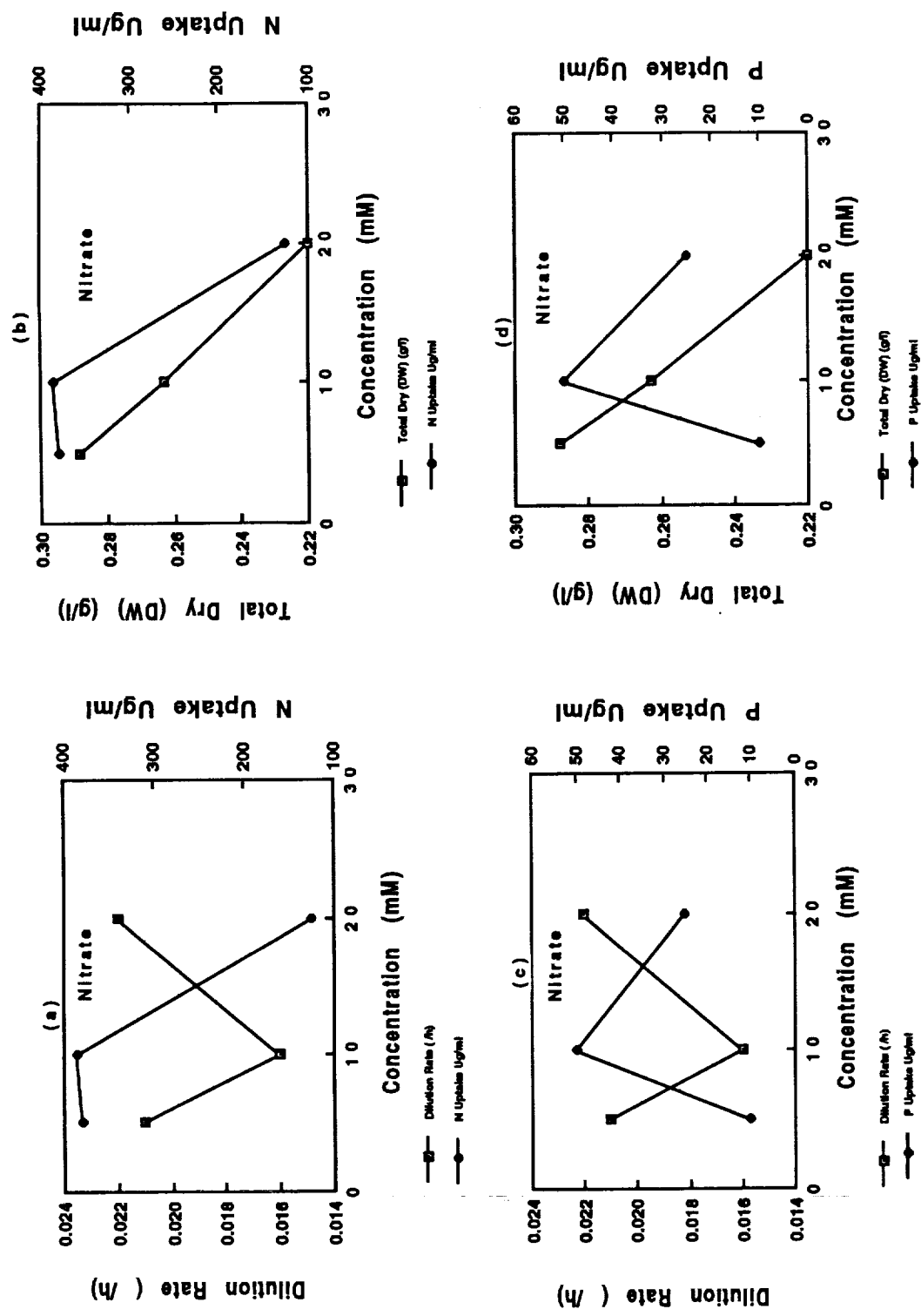


FIGURE 4: Growth Characteristics Of *Spirulina maxima* Maintained In Growth Medium, Of Different Concentrations Of Nitrate-Nitrogen, In Steady State Cultures (Light Intensity, 30 uE m<sup>-2</sup> s<sup>-1</sup>).

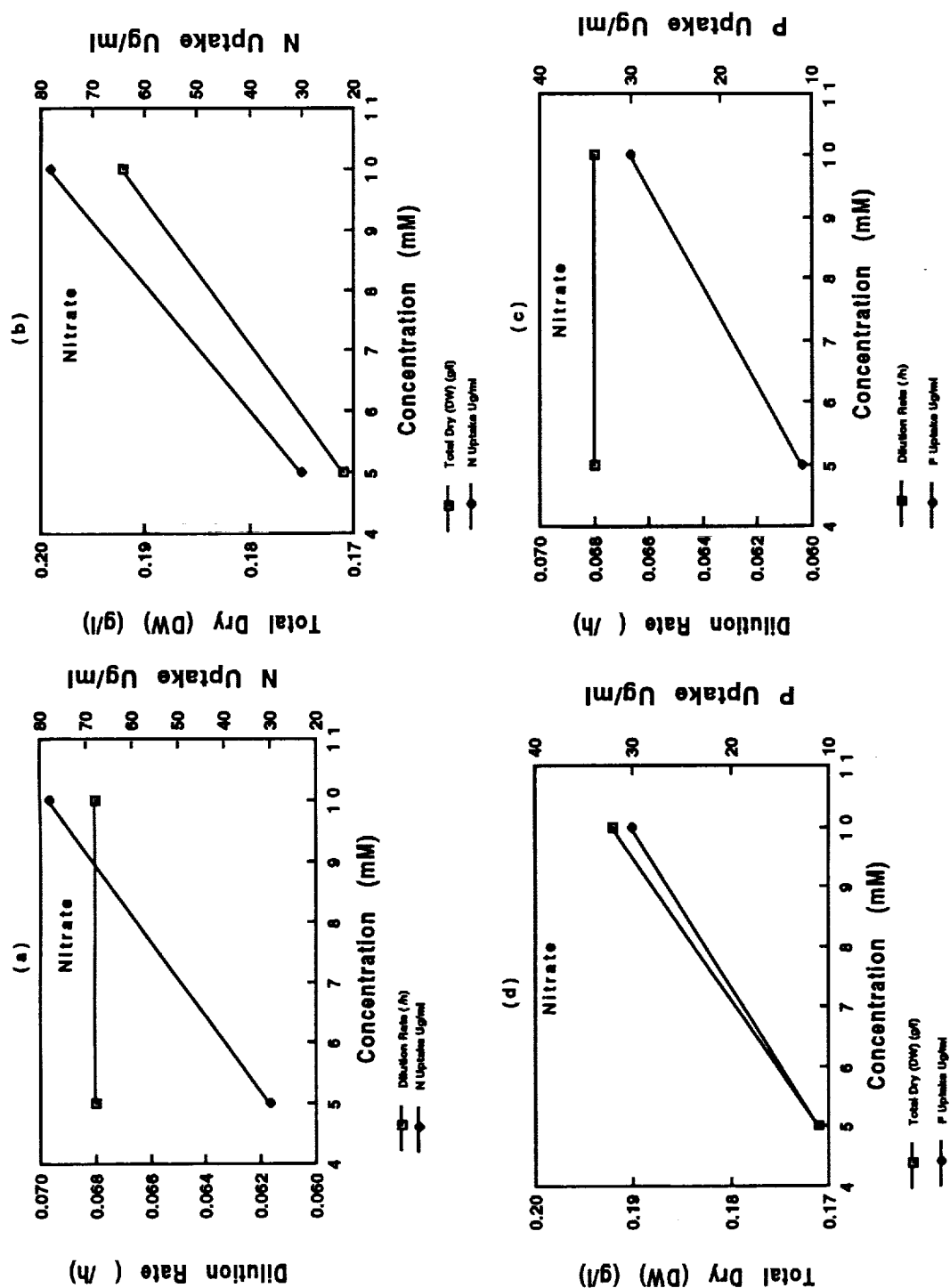


FIGURE 5: Growth Characteristics Of *Spirulina maxima* Maintained In Growth Medium Of Different Concentrations Of Nitrate-Nitrogen, In Steady State Cultures (Light Intensity, 100  $\mu$ E  $m^{-2} s^{-1}$ ).

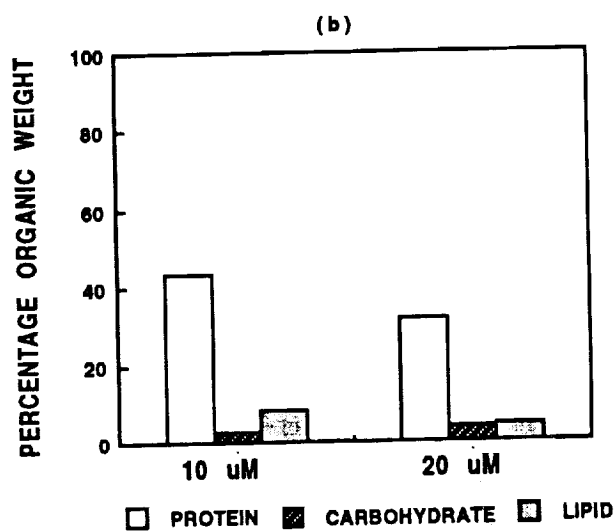
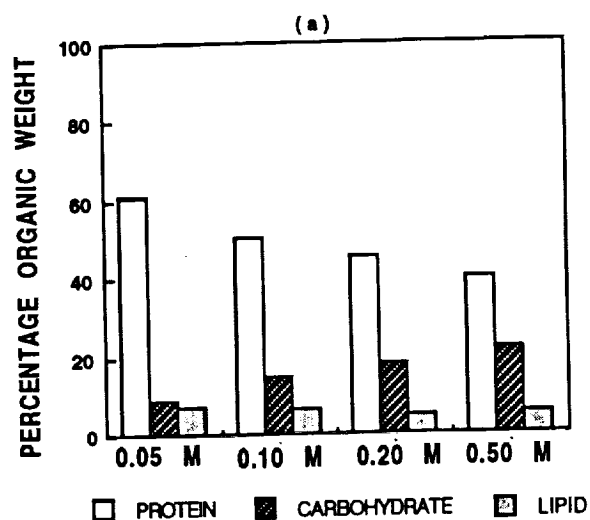


FIGURE 6: Chemical Composition Of *Spirulina maxima* Maintained In Growth Medium Of Different Concentrations Of Nitrate-Nitrogen, In Steady State Cultures (Light Intensity, 30 uE m<sup>-2</sup> s<sup>-1</sup>, 6a; 100 uE m<sup>-2</sup> s<sup>-1</sup>, 6b).

TABLE 3: Growth Characteristics Of *Spirulina maxima* Maintained In Growth Medium Of Different Concentrations Of Nitrate-Nitrogen In Steady State Cultures (Light Intensity 30 uE m-2 s-1).

O.D.	mM	Dilution Rate	Tot. Dry (Dw) (g/l)	Productivity (g/h/l)	Chlorophyll (mg/l)	Incident Light (cal/cm/h)	Absorbed Light a/v (cal/cm/h)	Growth Yield Y	Photosynth etic (g dw /cal)	Oxygen Evolved uM O2	% Organic wt. (afdw)		N Uptake Ug/ml	P Uptake Ug/ml	
											Protein	Carbohydrate			
0.48	5	0.021	0.288	0.0060	6.083	0.1475	0.1245	0.0481	28.37	1600	46.62	14.16	25.42	380.00	10.00
0.49	10	0.016	0.263	0.0042	6.285	0.1475	0.1236	0.0339	20.00	2500	48	19.6	20.21	385.00	50.00
0.48	20	0.022	0.022	0.0048	5.713	0.1475	0.1236	0.0388	22.89	1100	59	12.82	22.90	125.00	25.00



TABLE 4: Growth Characteristics Of Spirulina maxima Maintained In Growth Medium Of Different Concentrations Of Nitrate-Nitrogen In Steady State Cultures (Light Intensity 100 uE m-2 s-1).

Q.D.	mM	Dilu tion Rate (/h)	Tot. Dry (g/l) (Dw/)	Produc tivity (g/h/l)	Chloro phyll (mg/l)	Incident Light (cal/cm/h)	Absorbed Light a/v (cal/cm/h)	Growth Yield Y (g dw /cal)	Photo synth etic %	Oxygen Evolved uM O <sub>2</sub>	% Organic wt. (afdwt)			N Uptake Ug/ml	P Uptake Ug/ml
											Protein	Carbohydrate	Lipid		
0.283	5	0.068	0.171	0.0116	3.666	0.4935	0.4287	0.0270	15.93	100	31.84	3.65	4.2	30	11
0.283	10	0.068	0.192	0.0130	4.029	0.4935	0.4274	0.0304	17.93	700	43.30	2.65	8.17	78	30

### **III. Growth Characteristics of Spirulina Maintained Concentrations Of Growth Media Of Different Concentrations Of Phosphate-Phosphorus, At Steady State.**

Results are presented in Table 5 and Figures 7 and 8.

- . The dilution rate decreased with increasing the concentration of phosphate (Figure 7a).
- . The nitrogen uptake decreased with increasing the dilution rate or increasing the dry weight (Figure 7a and 7b). However the phosphorus uptake increased with the dilution rate and the dry weight (Figure 7c and 7d).
- . Increasing the concentration beyond 1mM did not improve the growth of the culture.
- . The chemical composition of the culture was enhanced by decreasing the phosphate to 0.1mM (Figure 8).
- . The evolved oxygen was somewhat higher in cultures treated with 0.5mM than in cultures treated with 0.1mM.

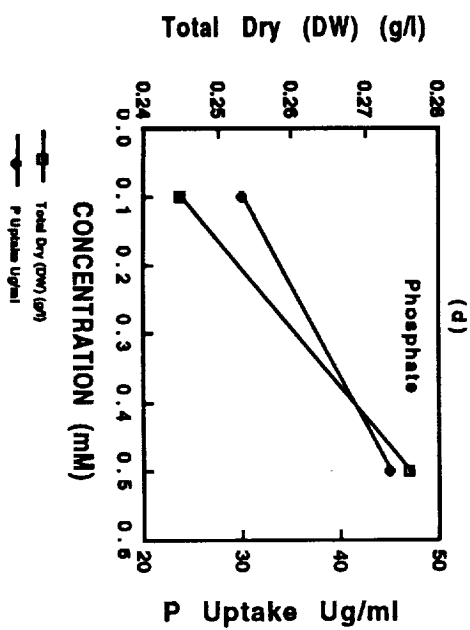
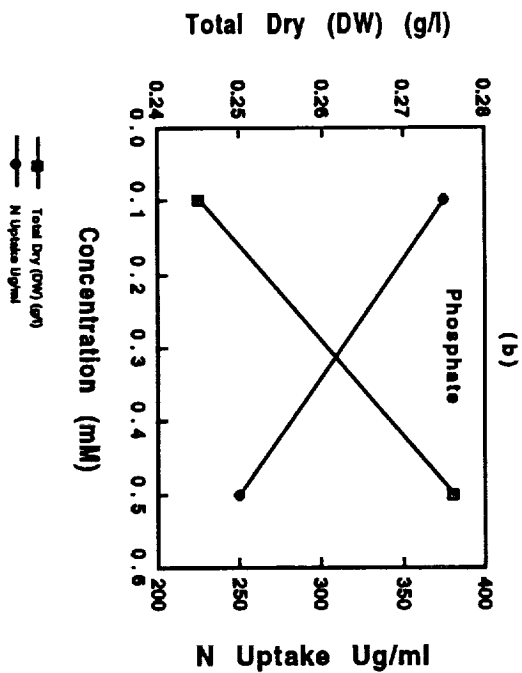
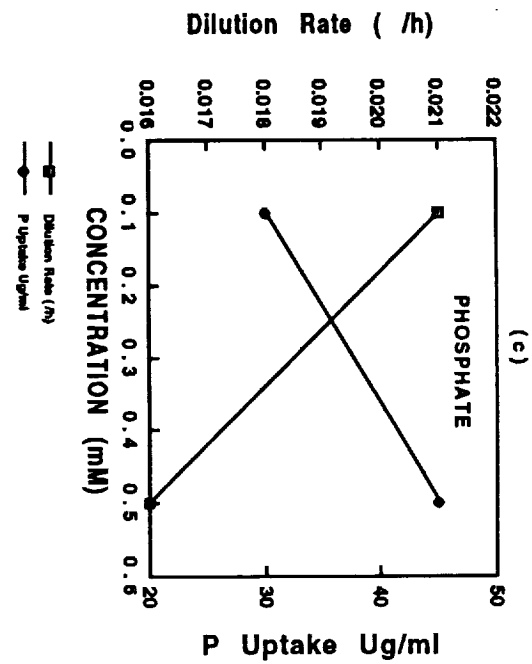
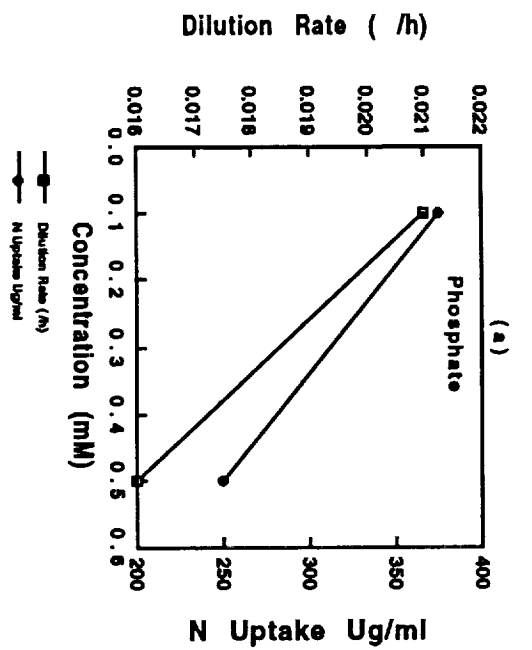


FIGURE 7: Growth Characteristics Of *Spirulina maxima* Maintained Of Different Concentrations Of Phosphate, In Steady State Cultures (Light Intensity, 30  $\mu\text{E m}^{-2} \text{s}^{-1}$ ).

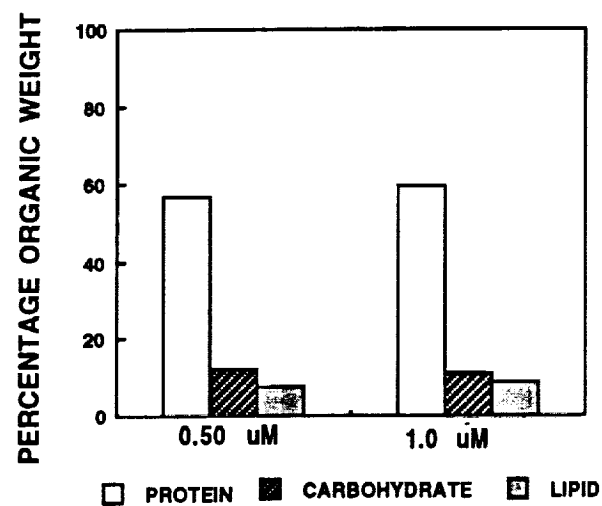


FIGURE 8: Chemical Composition Of Spirulina maxiam Maintained In Growth Medium Of Different Concentrations Of Phosphate, In Steady State Cultures (Light Intensity, 30 uE m<sup>-2</sup> s<sup>-1</sup>).

TABLE 5: Growth Characteristics Of Spirulina maxima Maintained In Different Concentrations Of Phosphate In Steady State Cultures (Light Intensity 30 uE m-2 s-1).

O.D.	mM	Dilu tion Rate ( /h)	Tot. Dry (g/l)	Produc tivity (g/h/l)	Chloro phyll (mg/l)	Incident Light (cal/ cm/h)	Absorbed Light a/v (cal/ cm/h)	Growth Yield (g dw /cal)	Photo synth etic % dw/h	Oxygen Evolved uM O2	% Organic wt. (afdw)			N Uptake Ug/ml	P Uptake Ug/ml
											Protein	Carbohydrate	Lipid		
0.49	0.5	0.016	0.276	0.0044	5.479	0.1475	0.1245	0.0353	20.82	1000	56.92	12.05	7.87	250.0	45.0
0.47	0.1	0.021	0.245	0.0051	6.486	0.1475	0.1236	0.0412	24.30	700	59.69	11.09	8.83	375	30

#### **IV. Growth Characteristics Of Spirulina Maintained In Growth Medium Of Different Concentrations Of Sodium Chloride At Steady-State.**

Results are presented in Table 6 and 7 and Figures 9,10, and 11.

. The total nitrogen uptake and dry weight increased with decreasing dilution rate and with increasing the concentration of Sodium Chloride up to 0.5M and started to decrease (Figures 9a and 9b).

. Cultures grown in high light intensity (Figure 10), showed a decrease in nitrogen uptake and dry weight. It should be mentioned that increasing the concentration of Sodium Chloride beyond 0.2 M was detrimental to the culture.

. The chemical composition of the culture showed a decrease in the amount of total protein while increase in the total carbohydrate (Figure 11a). Increasing the light intensity did not affect the total carbohydrate too much (Figure 11b). It should be mentioned that the cultures were deteriorated in high light intensity especially when the concentration of sodium chloride was increased.

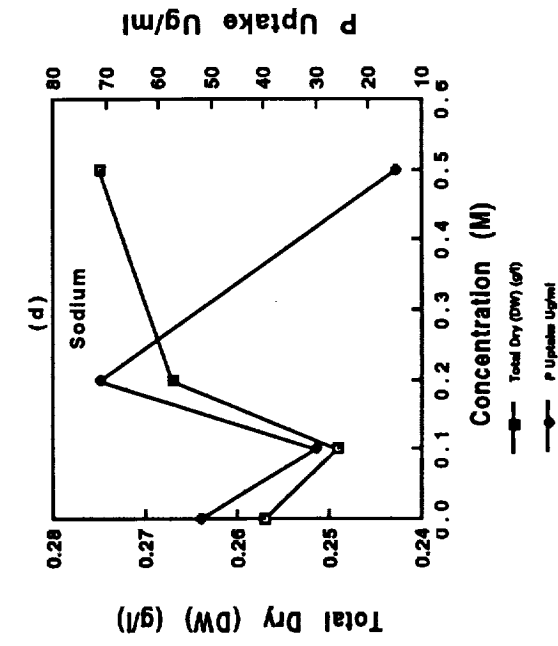
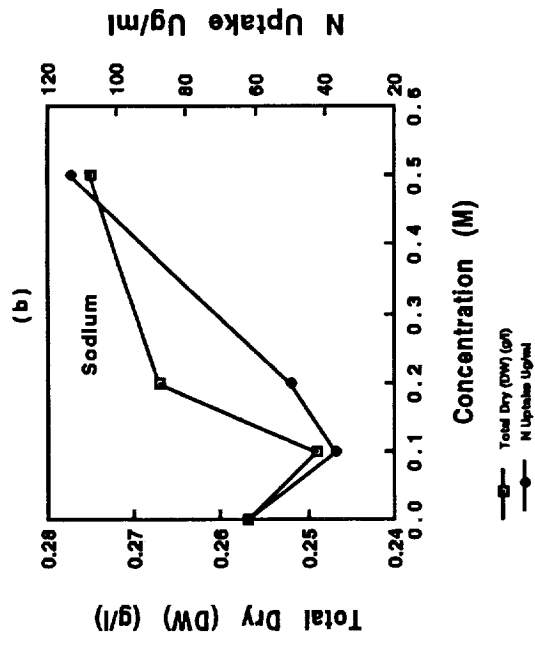
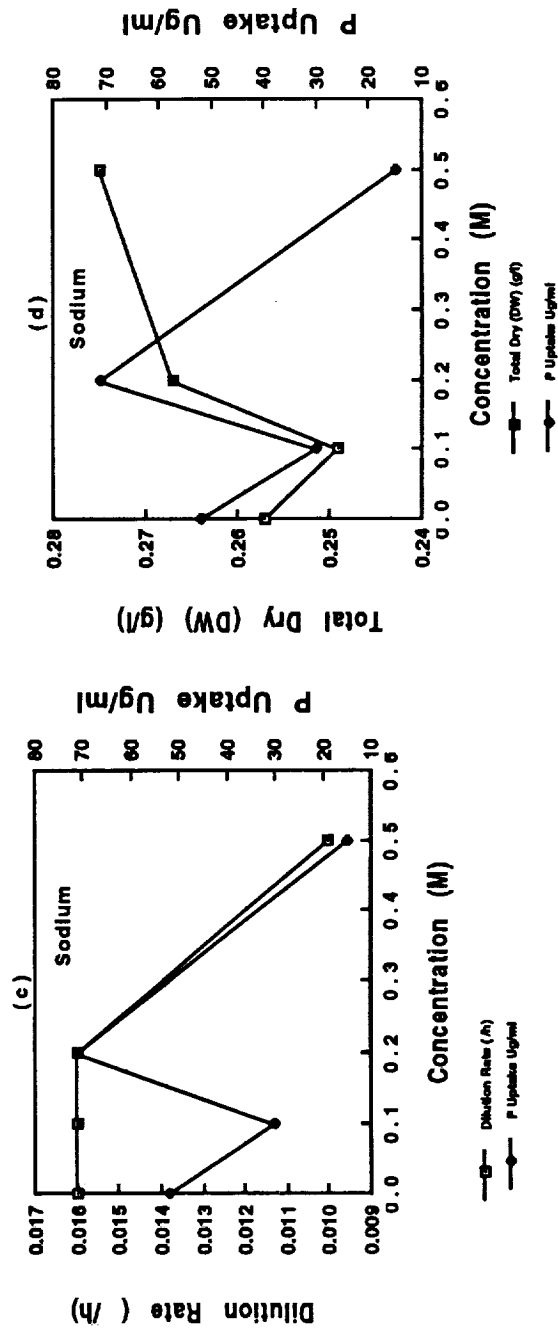
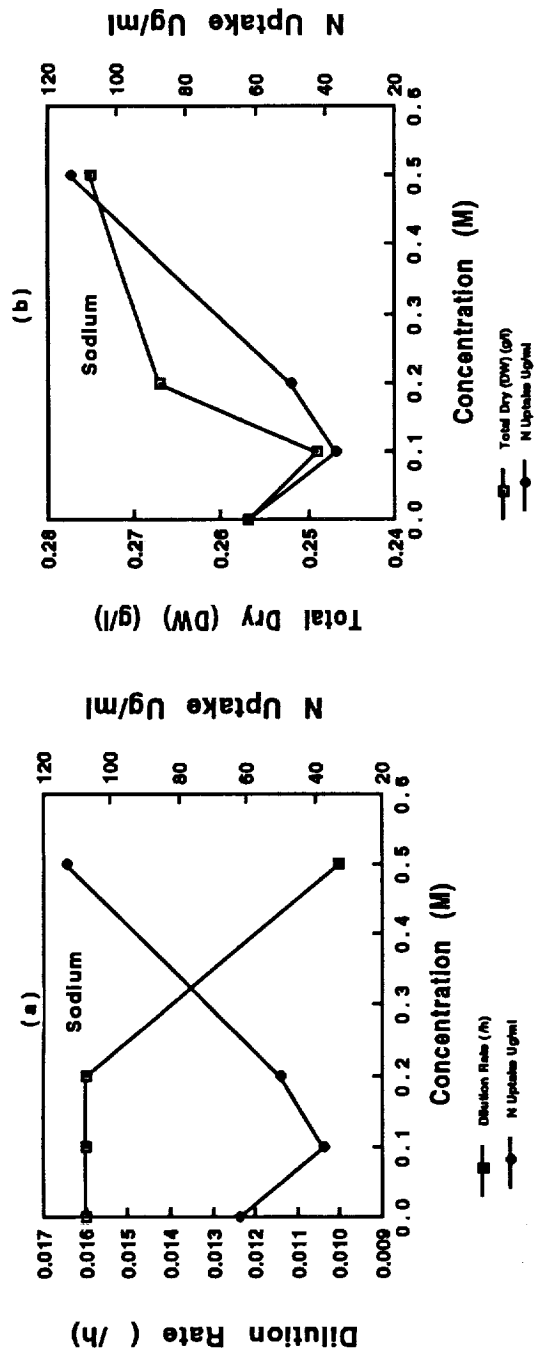


Figure 9: Growth Characteristics Of *Spirulina maxima* Maintained In Growth Medium Of Different Concentrations Of Sodium Chloride, In Steady State Cultures (Light Intensity, 30 uE m<sup>-2</sup> s<sup>-1</sup>).

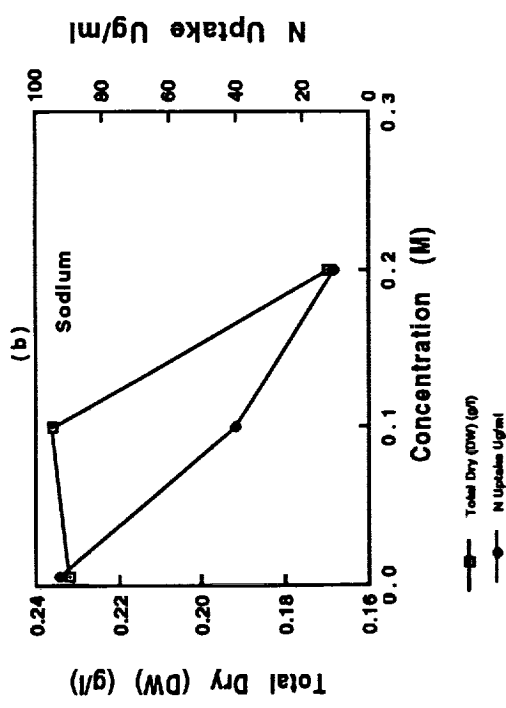
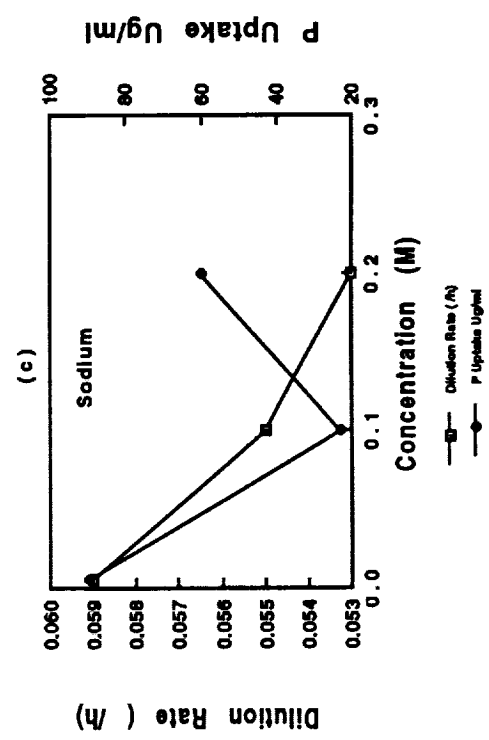
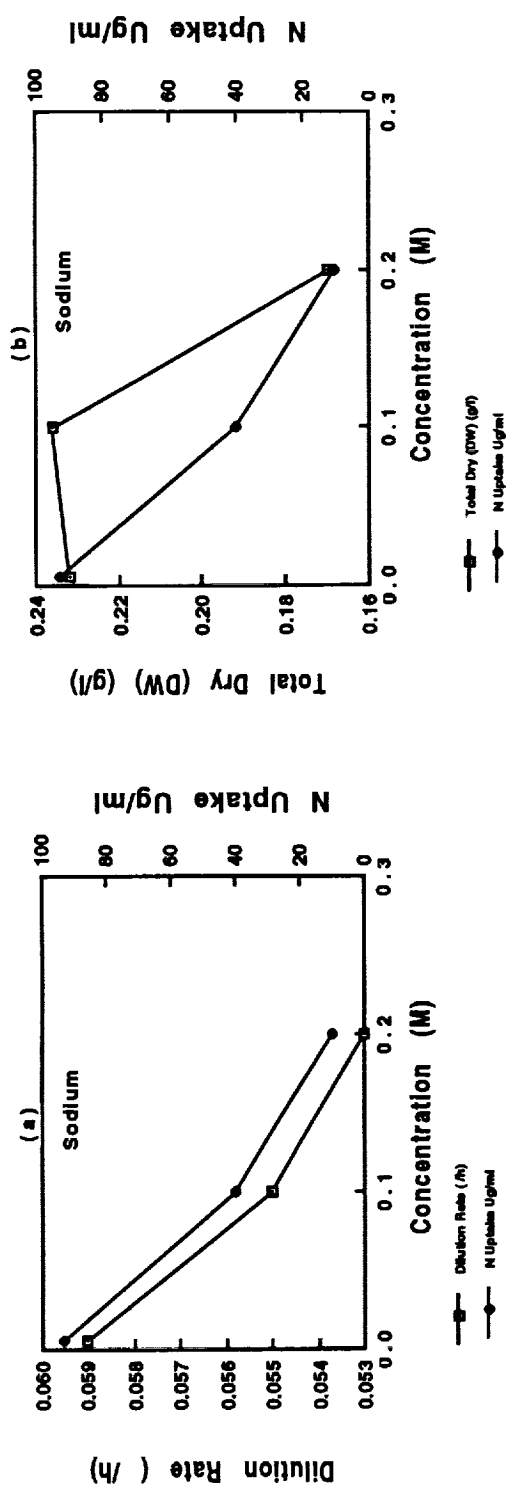


FIGURE 10: Growth Characteristics Of *Spirulina maxima* Maintained In Growth Medium Of Different Concentrations Of Sodium Chloride, In Steady State Cultures (Light Intensity, 100 uE m<sup>-2</sup> s<sup>-1</sup>).



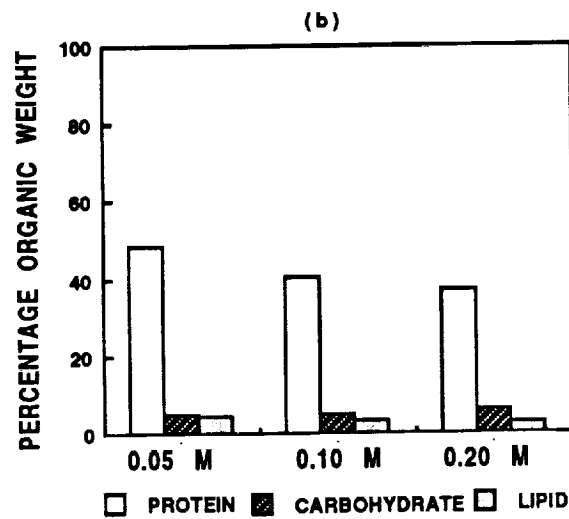
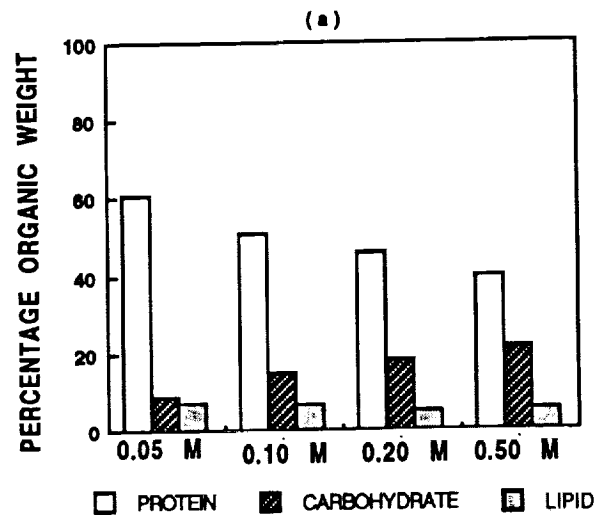


FIGURE 11: Chemical Composition Of Spirulina maxima Maintained In Different Concentrations Of Sodium Chloride, In Steady State Cultures (Light Intensity, 30 uE m<sup>-2</sup> s<sup>-1</sup>, 11a; 100 uE m<sup>-2</sup> s<sup>-1</sup> 11b).

TABLE 6: Growth Characteristics Of Spirulina maxima Maintained In Growth Medium Of Different Concentrations Of Sodium Chloride In Steady State Cultures (Light Intensity 30 uE m-2 s-1).

O.D.	M	Dilu tion Rate ( /h)	Tot. Dry (g/l)	Produc tivity (g/h/l)	Chloro phyll	inci dent Light cm/h)	Abso rbed Light a/v (cal/ cm/h)	Growth Yield Y (g dw /cal)	Photo synth etic %	Oxygen Evolved uM O2	% Organic wt. (afdw)		N Uptake Ug/ml	P Uptake Ug/ml
											Protein	Carbohydrate		
0.485	0.0	0.016	0.257	0.0041	6.607	0.1475	0.1228	0.0333	19.64	900	60.76	8.74	62	52
0.490	0.1	0.016	0.249	0.0039	6.48	0.1475	0.1249	0.0312	18.48	900	50.58	14.77	37	30
0.490	0.2	0.016	0.267	0.0042	8.46	0.1475	0.1241	0.0338	19.94	1300	45.72	18.38	50	71
0.485	0.5	0.010	0.275	0.0027	7.735	0.1475	0.1245	0.0217	12.80	2000	39.93	21.71	113	15

Table 7: Growth Characteristics Of Spirulina maxima Maintained In Growth Medium Of Different Concentrations Of Sodium Chloride In Steady State Cultures (Light Intensity 100 uE m-2 s-1).

O.D.	M	Dilu tion Rate ( /h)	Tot. Dry (DW) (g/l)	Produc tivity (g/h/l)	Chloro phyll (mg/l)	Incident Light (cal/cm/h)	Absorbed Light (cal/cm/h)	Growth Yield (g dw /cal)	Photo synth etic %	Oxygen Evolved uM O2	% Organic wt. (afdw)		N Uptake Ug/ml	P Uptake Ug/ml	
											Protein	Carbohydrate			
0.281	0.005	0.059	0.232	0.0137	3.344	0.4935	0.3776	0.0363	21.42	700	30.49	3.70	2.20	93	90
0.280	0.100	0.055	0.236	0.0130	2.618	0.4935	0.3602	0.0360	21.24	700	40.23	4.30	3.20	40	23
0.301	0.200	0.053	0.170	0.0090	2.739	0.4935	0.3689	0.0244	14.40	400	48.75	4.96	34.40	10	60

## CONCLUSIONS:

In terms of nutrient effect and light interaction

- . The productivity increased in experiments exposed to low light ( $30 \mu\text{E m}^{-2}\text{s}^{-1}$ )
- . Oxygen evolved and protein production were higher in cultures exposed to low light intensity
- . There was a relationship between nitrate concentration and the yield of the culture. Increasing the concentration of nitrate in the growth medium up to 20 mM was enough to produce a culture having the same chemical composition as that of complete medium. High light was inhibiting the yield of the culture.
- . Increasing the concentration of phosphate beyond 1mM did not improve the yield of the culture.
- . Increasing the concentration of sodium chloride in the growth medium did not affect the growth of the alga up to 0.1M but beyond that the culture started to be stressed. The response to stress appeared in high production of total carbohydrate on the expense of protein production. The oxygen production was also higher in cultures stressed with sodium chloride.

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